Workshop B
T Lymphocytes, Receptor Structure and Development

[1]Max-Planck-Institut für Immunbiologie, 7800 Freiburg, FRG, [2]Ludwig Institute for Cancer Research, Epalinges, Switzerland

B.1 Overlapping V_α and V_δ usage by TCR γ/δ expressing thymocytes

B. Arden[1], S. Wehr[1], H. R. Macdonald[2], and G. C. Miescher[2]

A subset of adult $CD4^-CD8^-$ thymocytes lacking the B2A2 antigen expresses α/β T-cell receptors (TCR) utilizing predominantly $V_\beta 8.2$. To a much lesser extent $B2A2^-$ double negative thymocytes also express γ/δ TCRs predominantly with $V_\delta 6$ genes that crosshybridize with $V_\alpha 7$ probes. These cells are phenotypically and developmentally distinct from the majority of adult γ/δ thymocytes which express the B2A2 antigen and preferentially use V_δ 5 and $V_\gamma 2$. With the polymerase chain reaction (PCR) we identified $V_\alpha 7.1$ as the gene segment that is utilized in the prominent $V_\delta 6$ transcript. In one hybridoma from the α/β TCR expressing $B2A2^-$ population we found an out-of-frame α-chain transcript of $V_\alpha 7.2$. We are trying to amplify by one-sided PCR RNA from the $B2A2^-$, α/β TCR^+ subset, to determine whether a particular V_α is found along with the dominant $V_\beta 8.2$. Whenever $V_\alpha 7.1$ and $V_\alpha 7.2$ occured in α-chain message, they were rearranged out of frame. These results suggest that these V gene segments can be rearranged both to J_α and J_δ gene segments, however, at a post-transcriptional level they are selected to be expressed exclusively as δ-chains. We also amplified RNA from newborn mice and frequently found δ-chain transcripts of functionally rearranged $V_\alpha 7.1$ and $V_\alpha 7.2$ gene segments. Our finding of dominant expression of $V_\alpha 7.1$ and $V_\alpha 7.2$ provides another example of preferential V_δ gene segment usage in thymocytes at different developmental stages. We are now trying to ablate these V gene segments by homologous recombination and thereby to assess their functional role.

[1]Max-Planck-Institut für Immunbiologie, 7800 Freiburg, FRG, and [2]Howard Hughes Medical Institute, National Jewish Center for Immunology, Denver, U.S.A.

B.2 Characterization of T cell hybrids with mutations in genes involved in T cell recognition and activation

Hans-Gerhard Burgert[1], Eckart Foerster[1], Philippa Marrack[2], and John Kappler[2]

We have developed a strategy to identify amino acids within the T cell receptor (TCR)/CD4 complex that are important for antigen, superantigen and MHC recognition. The system

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We have developed a strategy to identify amino acids within the T cell receptor (TCR)/CD4 complex that are important for antigen, superantigen and MHC recognition. The system

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includes random mutagenesis, a powerful selection step for enrichment of mutants with defects in antigen recognition or subsequent activation events and identification of mutations by the polymerase chain reaction and dideoxy sequencing. The method was applied to a $V_\beta 17_a^+$ $CD4^+$ T cell hybrid with dual specificity: It reacts with the antigen chicken ovalbumin (Ova) but also recognizes an unknown superantigen in conjunction with H2-E molecules. Mutants obtained upon selection on Ova presenting spleen cells fall into 2 major groups: 1) loss variants of TCR, CD4 or other molecules involved in T cell activation; 2) mutants with potential point mutations in one of those genes. In order to identify the particular gene affected we have analysed their ability to produce interleukin-2 under different experimental conditions. Work is in progress to identify potential point mutations of the TCR and/or CD4 genes by sequencing.

[1]Institut für Immunologie und Genetik, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, [2]Europäisches Laboratorium für Molekularbiologie, Meyerhofstr. 1, D-6900 Heidelberg, FRG

B.3 Tolerance and immunity to human C-reactive protein (CRP) in CRP-transgenic mice: A model system for an inducible self-protein

Rainer Döffinger[1], Ulrich Rüther[2], and Bruno Kyewski[1]

We study the immune response against human C-reactive protein in normal C57BL/6 mice and in mice transgenic for human CRP. The CRP-transgene is under the control of its human liver-specific regulatory sequences which tightly regulate its expression under the influence of acute phase inducers, e.g. interleukin-6. Transgene expression can be induced from undetectable levels (both at the m-RNA and protein level) to serum levels of about 100 µg/ml. This tight regulation of a "neo self-antigen" offers the possibility to study tolerance and immunity as a function of time- and dose-dependent antigen expression. We will report on the peptide specificity and T cell receptor usage of CRP-specific, $I\text{-}A^b$ restricted T-cell clones and *in vivo* self-antigen presentation at various induction levels.

Medizinische Hochschule Hannover, Klinik für Abdominal- und Transplantationschirurgie, D-3000 Hannover, FRG

B.4 Intestinal intraepithelial lymphocytes of the rat predominantly express the alpha/beta TCR but lack CD2

J. Fangmann, R. Schwinzer, and K. Wonigeit

Intestinal intraepithelial lymphocytes (IEL) represent a distinct cell population mostly composed of T cells with particular phenotypical characteristics. In this study the expression of the alpha/beta TCR (TCR2), CD2 and several other T-cell differentiation antigens on IEL of adult Lewis rats was analyzed. Single and two color flow cytometry analysis of isolated IEL was performed.

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Results: a) the vast majority (89 ± 3 %) of IEL reacted with mAb R73 detecting a monomorphic determinant on the rat alpha/beta TCR; b) less than 5 % of total IEL populations expressed the CD2 antigen; c) two color flow cytometry analysis revealed a prominent subset of CD4, CD8 double positive IEL (34 ± 13 %); d) virtually all IEL expressed the RT6 differentiation antigen present only on a subset of peripheral T cells in other lymphatic organs.

Conclusions: Rat IEL differ remarkably in TCR expression from mouse IEL which predominantly express the gamma/delta T cell receptor (TCR1). The identification of $TCR2^+CD2^-$ IEL as the predominant phenotype subset indicates that the expression of the TCR complex in the IEL populations is not linked to the simultaneous expression of the CD2 differentiation antigen.

Institut für Immunologie der Joh.-Gutenberg-Universität, D-6500 Mainz, FRG

B.5 Signals required for the proliferation of T_H1-lymphocytes

T. Germann and E. Rüde

According to their different pattern of lymphokines produced upon ligation of the T cell receptor (TCR) murine CD4-positive clones can be subdivided into T_H1 (IL-2, IFN-γ producer) and T_H2 (IL-4, 5, 6 producer) cell lines. Whereas the production of lymphokines is mainly regulated by the TCR-signal, additional "second signals" are required for the proliferation of the T cell lines. For T_H2-lymphocytes IL-1 functions as "second signal". In contrast, IL-1 fails to function as "second signal" for T_H1-lymphocytes due to the absence of IL-1 receptors on these lymphocytes. We have identified soluble mediator(s) termed T cell stimulating factor (TSF) in the supernatant of T_H1-lymphocytes stimulated by antigen presented by syngeneic spleen cells which are able to induce the proliferation (IL-2-receptor p55 chain re-expression) of T_H1-lymphocytes in an antigen-specific as well as an antigen-independent activation system. TSF was purified from such supernatants. We obtained a preparation free of detectable IL-1, IL-2, IL-4, IL-6, IL-9 (P40 TCGFIII) and TNF but is still active in inducing proliferation of T_H1-lymphocytes.

Institute of Medical Microbiology and Hygiene, Technical University of Munich, Trogerstr. 4A, 8000 Munich 80, FRG

B.6 Cyclosporine A prevents the generation of mature single-positive ($CD4^+$ $CD8^-$; $CD4^-$ $CD8^+$) alpha-beta T cell receptor (TCR) bearing thymocytes but spares double-negative gamma-delta T cell receptor bearing thymocytes

Klaus Heeg, Sylvia Bendigs, Pia Bader, and Hermann Wagner

Upon injection of Cyclosporine A (CsA) into newborn mice, the generation of single-positive ($CD4^+$ $CD8^-$, $CD4^-$ $CD8^+$) mature thymocytes is effectively prevented. As a consequence, the number of single-positive CD3 bearing T lymphocytes in peripheral lymphatic tissues (lymph node, spleen) becomes drastically (> 95 %) reduced. However, CD3-positive $CD4^-$ $CD8^-$

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lymphocytes are still detectable. FACS analyses using anti-gamma/delta TCR monoclonal antibodies revealed, that after CsA injection the number of $CD3^+$ $CD4^-$ $CD8^-$ gamma/delta T cell receptor bearing thymocytes remains unaltered. Peripheral as well as thymic $CD3^+$ $CD4^-$ $CD8^-$ lymphocytes could be expanded *in vitro* using anti-CD3 hybridoma cells plus IL-2. After 7 days of *in vitro* culture the majority of these cells retained the phenotype $CD3^+$ $CD4^-$ $CD8^-$ and beared gamma/delta TCRs. They displayed high lytic activity when tested in a lectin-facilitated ^{51}Cr-release assay. The results indicate that CsA allows the selective maturation of gamma/delta T cells while completely blocking the generation of alpha/beta T cells. It is speculated that gamma/delta T cells might play an important role in the pathogenesis of CsA induced graft versus host disease following syngeneic bone marrow transplantation.

[1]Department of Tumor Immunology, Institute of Immunology and Experimental Therapy, Wroclaw, Poland, and [2]Basel Institute for Immunology, Basel, Switzerland

B.7 Serological and molecular analysis of cell clones from the mouse fetal thymus stroma

Leszek Ignatowicz[1], Wojtek Swat[1], Yasushi Uematsu[2], and Pawel Kisielow[1]

Two cell clones from BALB/c 16d fetal thymus were established and characterized with regard to surface phenotype, rearrangements and expression of T-cell specific genes (TCR, IL-2R) and growth dependency on different growth factors.

Both clones were found to express Thy-1, Mac1, class I and II MHC antigens and low level of IL-2R. IFN-γ treatment highly increased expression of class II MHC antigens. No markers of early thymocytes (B14, J11D) or T cells (CD3, CD4, CD8) were detected.

TCR genes (α, β, γ, δ) in the clones analysed remained in germ line configuration and no mRNA specific for these loci was found. Specific transcripts from the IL-$2R_\alpha$ gene were detected.

Proliferation of the clones did not increase in response to stimulation by rIL-2, rIL-3 or rIL-4. Supernatants from cell cultures of these clones appeared to propagate growth of IL-3 dependent clones from bone marrow and inhibit growth of fetal thymocytes.

The results obtained suggest that both clones analysed represent the same population of fetal thymic stromal cells derived from the bone marrow. Interactions of these cell clones with immature thymocytes in *in vitro* co-culture are under investigation.

Institute of Immunology, University of Heidelberg, Im Neuenheimer Feld 305, D-6900 Heidelberg, FRG

B.8 T-cell receptor/CD3-signalling induces death by apoptosis in human T-cell receptor γδ-positive T-cells

Ottmar Janssen, Sebastian Wesselborg, Brigitte Hecht-Östreicher, and Dieter Kabelitz

Monoclonal antibodies (mAb) directed against the T cell receptor (TCR)/CD3 complex activate resting T cells. However, TCR/CD3 signalling induces death by apoptosis in

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immature ($CD4^+$ $CD8^+$) murine thymocytes and certain transformed leukemic T cell lines. Here we show that anti-TCR and anti-CD3 mAb induce growth arrest of cloned TCR$\gamma\delta^+$ T cells in the *presence* of IL-2. In the *absence* of exogenous IL-2, however, the very same anti-TCR/CD3 mAb stimulated $\gamma\delta^+$ clones to proliferation and IL-2 production. In the presence of exogenous IL-2, anti-TCR/CD3 mAb induced the degradation of DNA into oligosomal bands of approximately 200 bp length in cloned $\gamma\delta^+$ T cells. This pattern of DNA fragmentation is characteristic for the programmed cell death termed apoptosis. These results demonstrate that TCR/CD3 signalling can induce cell death in cloned $\gamma\delta$ T cells. In addition, this report is the first to show that apoptosis triggered by TCR/CD3 signalling is not restricted to $CD4^+$ $CD8^+$ immature thymocytes and transformed leukemic T cell lines but can be also observed with IL-2 dependent normal (i.e. TCR$\gamma\delta^+$) T cells.

Department of Medical Microbiology and Immunology, University of Ulm, Albert-Einstein-Allee 11, 7900 Ulm, FRG

B.9 Antigen reactivity of *Mycobacterium tuberculosis* activated gamma/delta T lymphocytes

Martin E. Munk, A. J. Gatrill, and S. H. E. Kaufmann

Mycobacterium tuberculosis infects host macrophages in this way producing a chronic infectious disease. T cells bearing the α/β T-cell receptor (TCR) are known to participate in the protection against *M. tuberculosis*, whereas only indirect evidence exists that T cells bearing the γ/δ TCR are involved in immunity to this pathogen. Our objective was to investigate the *in vitro* response of γ/δ T lymphocytes to *M. tuberculosis* and other bacteria. Peripheral blood T lymphocytes from healthy donors were stimulated *in vitro* and a marked expansion of the γ/δ T-cell population was observed 7 to 10 days after stimulation with *M. tuberculosis* (15/21 donors), *M. leprae* (6/8), *Staphylococcus aureus* (1/6), group A streptococci (7/9), or *Listeria monocytogenes* (3/9) as assessed with anti-TCR 1-FITC antibody by FACScan analysis. *M. tuberculosis* activated γ/δ T lymphocytes expressed IL-2 receptors and secreted IL-2 upon restimulation with *M. tuberculosis*. *M. tuberculosis* directed cytolytic activity of γ/δ T lymphocytes was demonstrated in the following ways: (1) γ/δ T cells lysed *M. tuberculosis* primed but not unprimed targets; (2) high concentrations of anti-TCR 1 mAb facilitated killing of unprimed target cells; (3) low doses of anti-TCR1 mAb blocked killing of primed targets; (4) γ/δ T cells after activation with *M. tuberculosis* or with group A streptococci, respectively, lysed targets primed with the homologous agents only. We conclude that, upon contact with mycobacteria and perhaps other microorganisms, γ/δ T cells are activated which can contribute to immunity via IL-2 secretion and specific target cell lysis.

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Institute of Immunology, University of Heidelberg, Im Neuenheimer Feld 305, 6900 Heidelberg, FRG

B.10 CD2 can be induced to functionally associate with the TCR on γδ⁺ T cells

KLAUS PECHHOLD, SEBASTIAN WESSELBORG, OTTMAR JANSSEN, and DIETER KABELITZ

T cell receptor (TCR) αβ⁺ as well as γδ T-cells can be activated by crosslinking their respective TCR/CD3 complexes. In addition, triggering of CD2 by a combination of mAb directed at different epitopes of the CD2 molecule are also known to result in T-cell activation in cases where a functional TCR/CD3 complex is expressed on the cell surface. We were able to demonstrate that CD2 can be induced to functionally and possibly physically associate with the TCR/CD3 at least in γδ⁺ T-cells. In view of the observation that immobilized anti-CD2 mAb trigger γδ⁺ T-cells to proliferation and display of cytolytic activity, we investigated a possible interdependence of CD2 and TCR/CD3 surface molecules during the activation process. Cytofluorometric analyses clearly showed a failure of certain anti-γδTCR/CD3 mAb to bind after preincubation with anti-CD2 mAb. This is probably due to induction of conformational changes in the TCR/CD3 complex. Co-immunoprecipitation studies and co-modulation studies will be performed in order to distinguish a functional or physical association. Such conformational changes in TCR/CD3 molecules are regarded to be necessary for productive signal transduction.

[1]Institute of Medical Microbiology and Hygiene, Technical University of Munich, 8000 Munich, FRG, and [2]Department of Medical Microbiology and Immunology, University of Ulm, 7900 Ulm, FRG

B.11 Primary responses of human T cells to mycobacteria: a frequent set of γ/δ T cells are stimulated by protease-resistant ligands

KLAUS PFEFFER[1], BERND SCHOEL[2], HEINZ GULLE[2], S. H. E. KAUFMANN[2], and HERMANN WAGNER[1]

Studies in the last years revealed, besides α/β T cells, the existence of a second T-cell population which expresses a T cell receptor heterodimer (TCR) composed of γ and δ chains. Up to the present time several features of these γ/δ T cells including their biological function and antigen specificity remain ill-defined.

In our study T lymphocyte subsets expressing either α/β or γ/δ TCR were selected from human peripheral blood T cells and proliferative responses to mycobacteria and mycobacterial components were determined. Whereas both α/β and γ/δ T cells responded to intact and lysed mycobacteria, protease digestion abolished the stimulating activities for α/β T cells confirming that α/β T cells respond to protein components. In contrast, components recognized by γ/δ T cells proved resistant to protease digestion. In limiting dilution studies, frequencies of proliferating γ/δ T cells remained virtually unaltered by protease treatment of stimulating lysates, while those of α/β T cells became almost undetectable. Furthermore, only few γ/δ T cells responded to the 65 kD heat shock protein. Our data indicate that – unlike α/β T cells- γ/δ T cells preferentially respond to non-peptide components within mycobacterial lysates.

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[1]Department of Medical Microbiology and Immunology, University of Ulm, Albert-Einstein-Allee 11, 7900 Ulm, and [2]Institute of Medical Microbiology and Hygiene, Technical University, 8000 Munich, FRG

B.12 Primary responses of human T cells to mycobacteria: low molecular weight fractions stimulate only gamma/delta T cells, not alpha/beta T cells

Bernd Schoel[1], Heinz Gulle[1], Klaus Pfeffer[2], Hermann Wagner[2], and Stefan H. E. Kaufmann[1]

Mononuclear cells from the peripheral blood of normal donors were negatively selected into α/β T cells and γ/δ T cells, and afterwards their antigen reactivities were studied using preparations of *Mycobacterium tuberculosis* H37Rv. Mycobacterial lysates were separated by 2D-electrophoresis into 480 distinct fractions. While α/β T cells gave strong responses to numerous fractions, γ/δ T cells reacted to virtually none of them. Because both populations responded to whole lysates, it could be possible that electrochemical and/or size features of the ligands for γ/δ T cells were out of the range of the 2D-electrophoresis system. Therefore, we separated lysates according to size by gel filtration techniques. The ligands recognized by α/β T cells resided in the 20–80 kD fractions, whereas those recognized by γ/δ T cells resided in fractions of less than 3 kD. In these low molecular weight fractions, responses of γ/δ T cells were not detectable. These experiments indicate that γ/δ T cells and α/β T cells recognize antigens of different physicochemical nature.

Institute of Immunology, University of Heidelberg, Im Neuenheimer Feld 305, 6900 Heidelberg, FRG

B.13 Frequency and specificity of γδ $^{+}$ cytotoxic lymphocyte precursors activated by allogeneic or autologous stimulator cells

Susanne Schondelmaier, Anke Bender, Marcia Liane da Silva Lobo, and Dieter Kabelitz

The physiological significance of $\gamma\delta^+$ T cells is still largely unknown. Here we have used limiting dilution (LD) culture systems to investigate the frequency and specificity of alloantigen-reactive δ^+ cytotoxic lymphocyte precursors. $\gamma\delta^+$ T cells were isolated from E rosette-separated T cells by depletion of $CD4^+$, $CD8^+$, $CD16^+$ and $CD56^+$ cells. Freshly isolated $\gamma\delta^+$ T cells did not exert NK activity but responded under LD conditions in high frequency (1/400 to 1/4500) to allogeneic or autologous stimulator cells. The majority of developing $\gamma\delta^+$ cytotoxic effector cells lysed both autologous and HLA-mismatched allogeneic Con A blast targets and thus did not display allospecificity. In contrast, E-rosette purified "total" T cells clonally developed into allospecific CTL colonies when stimulated by HLA-mismatched stimulator cells. We conclude that specific recognition of allelic HLA class I or class II molecules is not a frequent property of $\gamma\delta^+$ T cells.

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Klinik für Abdominal- und Transplantationschirurgie, Medizinische Hochschule Hannover, 3000 Hannover, FRG

B.14 Preferential activation of CD45RA$^+$ T cells by a monoclonal antibody to the α/β T cell receptor

R. Schwinzer, H. J. Schlitt, and K. Wonigeit

Monoclonal antibodies (mAb) detecting different CD3/T cell receptor (TCR) epitopes are useful tools to study the question whether the activation of distinct T cell subsets can be controlled on the level of functionally different compartments in the CD3/TCR complex. To this end we compared the stimulatory activity of mAb OKT3 (IgG2a; anti-epsilon chain of CD3) with that of mAb BMA 031 (IgG2b) directed to a constant epitope on the α/β TCR in peripheral blood mononuclear cells (PBMC), and in isolated CD4$^+$ (helper/inducer), CD8$^+$ (cytotoxic/suppressor), CD45RA$^+$ (naive), and CD45RO$^+$ (memory) T cells.

Results: (a) Stimulation of PBMC by both, OKT3 (15 ng/ml) and BMA 031 (1.5 µg/ml) resulted in a comparable proliferative response. (b) Compared to PBMC, CD45RA$^+$ cells showed a markedly enhanced proliferative response to BMA 031 stimulation whereas the reactivity to OKT3 was slightly decreased. (c) The CD45RO$^+$ subset responded upon OKT3 stimulation with IL-2 secretion and proliferation. In contrast, BMA 031 stimulation of CD45RO$^+$ cells led to an IL-2 responsive state but neither to IL-2 secretion nor to significant proliferation. (d) Isolated CD4$^+$ and CD8$^+$ cells responded similarly to OKT3 and BMA 031 stimulation. Compared to unseparated PBMC there was an increased proliferation of CD8$^+$ cells and a decreased proliferative response of CD4$^+$ cells.

Conclusion: In vitro stimulation of lymphocytes by the anti-γδ$^+$ TCR mAb BMA 031 results in a preferential activation of the CD45RA$^+$ subset whereas CD45RO$^+$ cells are nearly unresponsive. Since the CD3 mAb OKT3 stimulates both subsets it is suggested that the activation of a distinct functional program in CD45RA$^+$ and CD45RO$^+$ cells may be controlled on the level of functionally different compartments in the CD3/TCR complex.

Dept. of Tumor Immunology, Institute of Immunology and Experimental Therapy, Wroclaw, Poland

B.15 Fetal thymic stromal cell clone induces deletion of double positive CD4$^+$ CD8$^+$ thymocytes

Wojtek Swat, Leszek Ignatowicz, and Pawel Kisielow

Fetal and newborn thymocytes were cocultured with cell clone D6 with properties of thymic macrophages established from murine 16 days fetal thymus. After 1 h of incubation, thymocytes (mainly CD4$^+$ CD8$^+$) form rosettes around D6 cells.

Using FACS analysis we found that prolonged incubation induces death of CD4$^+$ CD8$^+$ thymocytes, resulting in the lower expression of CD4, CD8, HSA and Thy1 antigens as compared to the expression of these antigens on thymocytes cultured alone.

Direct cell-to-cell contact is probably needed during this process, as supernatant of D6 cells is ineffective. Also it appears that fixation of D6 cells does not change their ability to induce death of CD4$^+$ CD8$^+$ thymocytes, so other mechanisms than phagocytosis should be considered. We suggest that the observed process of deletion of double positive thymocytes by fetal thymus stromal cells may be involved in the mechanisms of T cell selection in the thymus.

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[1]Department of Immunology and Cell Biology, Forschungsinstitut Borstel, D-2061 Borstel, FRG, and [2]Department of Pathology, University of Würzburg, D-8700 Würzburg, FRG

B.16 Inhibitory effects of anti-CD26 monoclonal antibodies on mitogen- and antigen-driven T-lymphocyte proliferation

A. J. Ulmer[1], T. Mattern[1], A. C. Feller[2], and H.-D. Flad[1]

CD26 is an activation antigen on the surface of human T lymphocytes, which is known to be the dipeptidyl peptidase IV (DPP IV). This activation marker is already present on resting T-cells (10 %–60 %) and is *de novo* expressed after activation on both CD26-negative and CD26-positive T-cells. To investigate whether CD26 might be involved in the processes of T-cell activation we have studied the effects of monoclonal anti-CD26 antibodies on the activation and proliferation of T lymphocytes after mitogenic or antigenic stimulation. Two different CD26 antibodies, anti-DPP IV (TII 19-4-7) and anti-Tal (4EL1C7) were used. Both antibodies did not inhibit the enzymatic activity of DPP IV. Anti-DPP IV as well as anti-Tal could inhibit the proliferation of T cells after mitogenic or antigenic stimulation in a dose-dependent manner. By addition of purified DPP IV to this system the inhibitory effect of the antibodies could be abrogated. In contrast to the proliferation, the release of interleukin-2 and the expression of various activation antigens (CD25, HLA-DR, and Ki-67) were not affected. We conclude that CD26 is required by activated T cells for progressing through the cell cycle rather than for an activation of the cells. Its function may not necessarily be connected with its enzymatic activity.

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Institute of Immunology, University of Heidelberg, Im Neuenheimer Feld 305, D-6900 Heidelberg, FRG

B.17 Selective stimulation of TCRγδ$^+$ human T cells via CD2 (T11.1)

Sebastian Wesselborg, Ottmar Janssen, Klaus Pechhold, and Dieter Kabelitz

T cells can be activated via the T cell receptor (TCR)/CD3 complex or alternatively via CD2. The CD2 (T11) molecule is characterized by 3 functional epitopes. Soluble monoclonal antibodies (mAb) against T11.2 and T11.3 exert a mitogenic effect on T cells whereas the T11.1 epitope is mainly involved in cell adhesion as it is the natural ligand for LFA-3. MAb against T11.1 are only mitogenic in concert with submitogenic concentrations of anti-T11.2 plus anti-T11.3. Recently it has been reported that cloned TCRγδ-positive T cells could be triggered by anti-T11.1 mAb alone to kill. We demonstrate that several mAb directed against T11.1 (OKT-11, 1OT-11, 9E8) stimulate cloned TCRγδ-positive T cells but not TCRαβ-positive T cells to proliferate and to secrete IL-2 in the absence of accessory cells, and to kill P815 target cells. Killing of P815 was achieved by addition of soluble mAb while proliferation and IL-2 production depended on immobilization of T11.1 mAb. Taken together, our data indicate that the involvement of CD2 in direct activation might be one of the major differences between TCRαβ-positive and TCRγδ-positive T cells with respect to activation requirements.

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Max-Planck-Institut für Immunbiologie, Stübeweg 51, 7800 Freiburg, FRG

B.18 Deviations in thymocyte development induced by divalent and monovalent ligation of the α/β T-cell receptor and by its cross-linking to CD8

Pedro Yachelini and Klaus Eichmann

Fetal thymic organ cultures (FTOC) were tested as a model system to induce, in a polyclonal fashion, negative and positive thymic selection events, using three different antibody reagents: anti-$V_\beta 8$ (F23.1), anti-Lyt-2.2 (19/178) and the quadroma derived bifunctional antibody HPHT-2, carrying one binding site of each. This antibody served also as a monovalent anti-$V_\beta 8$ reagent in FTOC from Lyt-2.1 mouse strains. Antibody 19/178 had no effect at the relatively low concentrations used. F23.1 and HPHT-2 suppressed the development of $CD4^+$ $V_\beta 8^+$ and $CD8^+$ $V_\beta 8^+$ thymocytes at concentrations giving rise to $V_\beta 8$ occupancies from about 2 % upwards. $V_\beta 8$ suppression occurred upon divalent and monovalent $V_\beta 8$ binding and was not significantly influenced by $V_\beta 8$-CD8 cross-linking. This suggests that CD8 is not essential in transmembrane signalling for clonal deletion. At lower antibody concentrations, $V_\beta 8$-CD8 cross-linking by HPHT-2 – but not divalent and monovalent $V_\beta 8$ ligation – induced an increased proportion of $V_\beta 8^+CD8^+$ cells within an unchanged number of total $V_\beta 8^+$ cells. The effect was quantitatively of borderline significance but reproducible. These results are compatible with the hypothesis that cross-linking of the αβ T-cell receptor and CD8 on the thymocyte surface provides a maturation signal resulting in loss of CD4 from $CD4^+CD8^+$ immature thymocytes.

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Workshop C
T Lymphocytes, Functional Specificity and MHC

Max-Planck-Institut für Immunbiologie, Stübeweg 51, 7800 Freiburg, FRG

C.1 Molecular approaches to the study of the function of MTSP-1

J. Chluba-de Tapia and M. M. Simon

MTSP-1 is a *m*urine *T* cell specific *s*erine *p*rotease. This proteolytic enzyme is expressed in activated $CD8^+$ and $CD4^+$ lymphocytes only. MTSP-1 is stored in cytoplasmic granules together with other proteases and perforin and is released upon antigenic stimulation. The function of MTSP-1 is studied by a molecular genetical approach using antisense-oligonucleotides and antisense RNA-techniques to inhibit the expression of the enzyme in T cell lines. Being aware that not all cell types can take up oligonucleotides in normal culture medium, we first tested the uptake of radioactively labeled oligonucleotides by 17 different $CD8^+$ and $CD4^+$ T cell lines. Only two MTSP-1^-, $CD4^+$ but none of the $CD8^+$ T cell lines showed uptake under these conditions. By optimizing the conditions for transfer of oligonucleotides into cells we finally succeeded in high uptake of radioactively labeled oligonucleotides in MTSP-1^+, $CD8^+$ T cells. The effects of antisense-oligonucleotides on MTSP-1 are being tested under these new conditions. For the antisense-RNA approach three expression vectors have been constructed with different fragment lengths of the MTSP-1 cDNA inserted in an inverted orientation downstream of the Rous-Sarcoma virus LTR. These constructs will be used for stable expression in a MTSP-1^+, $CD8^+$ T cell line. The expression and correct splicing of the antisense-RNA are detected by polymerase chain reaction. We hope by these approaches to define the biological function of MTSP-1.

Klinische Forschergruppe für Rheumatologie und Abteilung Rheumatologie und Klinische Immunologie, Medizinische Universitätsklinik Freiburg, Mooswaldallee 1-9, 7800 Freiburg, FRG

C.2 Differential antigen processing and presentation by various cell lines transfected with the same major histocompatibility complex (MHC) class II genes

Claudia Daubenberger, Bernhard Lang, and Inga Melchers

Various antigen presenting cells (APC) can process and present a given antigen in different ways. Therefore the resulting T cell response might differ according to the APCs used. To test

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Workshop C
T Lymphocytes, Functional Specificity and MHC

Max-Planck-Institut für Immunbiologie, Stübeweg 51, 7800 Freiburg, FRG

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this hypothesis we cotransfected the major histocompatibility complex (MHC) class II genes DRA and DRB1*0401 encoding the specificity for the human leukocyte antigen (HLA)DR4/Dw4 by electroporation. The host cells were P388D1 (a mouse macrophage-like cell line), U 937 (a human monocytic lymphoma cell line) and L cells (a mouse fibroblast cell line). As shown by Northern blot and FACS-analysis, the transfectants transcribe the genes and express their products properly. The processing capacities and antigen presenting properties of these transfectants are compared with EBV-transformed B cell lines and peripheral blood lymphocytes expressing the same HLA-DR genes. The test system consists of tetanus toxoid (TT) as the antigen and TT-specific DR4/Dw4 restricted T cells. The results show that transfectants differ greatly in processing and presentation of TT.

Institut für Immunologie der Joh.-Gutenberg-Universität, D-6500 Mainz, FRG

C.3 The apparent MHC-unrestricted cytotoxicity of T cell clone 10BK.1 is based on the MHC-restricted self-presentation of the specific antigen ovalbumin

T. Dick and A. B. Reske-Kunz

The $CD8^+$, $CD4^-$ T cell clone 10BK.1 was obtained from lymph node T cells of ovalbumin-immunized (B10 × B10.BR)F1 mice. Clone 10BK.1 recognizes the ovalbumin peptide 258-276 together with H-$2K^b$ molecules. Upon coculture with the antigen in the absence of added antigen-presenting cells 10BK.1 cells react by the production of lymphokines and by proliferation suggesting self-presentation of the antigen by the T clone cells. In addition clone 10BK.1 exhibits cytotoxic capacity. Syngeneic as well as allogeneic targets and even a human target were killed when the antigen was present. We explain this finding by implicating a two-step process. In the first step, 10BK.1 cells present the antigen to one another in an MHC-restricted manner and thereby get activated. In a second step, activated 10BK.1 cells are able to kill bystander cells in an MHC-unrestricted way.

Supported by the Deutsche Forschungsgemeinschaft, SFB 311, Mainz.

Max-Planck-Institut für Immunbiologie, Stübeweg 51, 7800 Freiburg, FRG

C.4 Differential expression of the T-cell associated proteases MTSP-1 and granzymes B-G in *in vitro* and *in vivo* derived activated mouse lymphocytes

K. Ebnet, J. Chluba-de Tapia, and M. M. Simon

Cytolytic T cells mediate their regulatory and effector functions with granule-associated proteins that are released upon activation of the lymphocyte via the TCR/CD3-complex. Recently, in addition to a pore-forming protein (perforin/cytolysin) a set of seven serine proteases, termed MTSP-1 (murine T cell specific serine protease 1) and granzymes B-G has

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been isolated from cloned $CD8^+$ T-cell lines. However, only for one of these structures, i.e. MTSP-1, significant amidolytic activity has been found. Moreover, there is indirect evidence for the involvement of MTSP-1 in T-cell mediated functions such as cytolysis, regulation of B-cell responses and extravasatation. To get more information on the physiological relevance of the seven proteases we have analyzed their expression using the polymerase chain reaction. To this end we tested a panel of different T cell populations with different phenotypes including long term cultured lines (TLC) as well as T cells sensitized *in vivo* either to lymphocytic choriomeningitis (LCM) virus or to I-A alloantigen, or polyclonally activated *in vitro* by ConA. From all T cell populations studied, only the $CD8^+$ and $CD4^+$ TLC expressed messenger RNA (mRNA) specific for all seven proteases, though at different levels. In contrast, in both *in vivo* sensitized $CD8^+$ and $CD4^+$ T cells and in *in vitro* activated T-cell populations only mRNA specific for MTSP-1 and granzyme B and to a lesser extent for granzyme C was found and neither of the granzymes D-G coding mRNAs were detectable. These results indicate that under physiological conditions effector T cells express MTSP-1 and granzymes B (and C) mainly and that for the induction of granzymes D-G different signals are required. The data suggest that, if at all, only MTSP-1 and granzymes B and C may play a role in T-cell mediated functions *in vivo*.

Abt. Immungenetik, Universität Göttingen, D-3400 Göttingen, FRG

C.5 Effect of heat shock on major histocompatibility complex class I-restricted cell-mediated lympholysis

G. Geginat, L. Heine, E. Rothermel, and E. Günther

We used rat major histocompatibility antigen (RT1)-specific and minor histocompatibility antigen-specific cytotoxic T lymphocytes (CTL) for lysis of allogeneic and syngeneic ^{51}Cr-labeled concanavalin A (ConA) blasts, which were either heat shock-treated or not. Heat shock consisted of culture at 44 °C for 30 min followed by a 6 h recovery phase, and resulted in expression of various heat shock proteins, e.g. hsp68. After heat shock of the target cells we observed an elevation of specific lysis in RT1 and especially minor histocompatibility antigen-specific cell-mediated lympholysis (CML). Syngeneic control target lysis showed no change after heat shock. To further analyse this effect we used the rat myeloma cell line Y3, which we have found to be hsp68-defective, and which is derived from the same inbred rat strain as the lymphoblast target cells. With Y3 targets we observed an inversion of the heat shock effect described above for ConA blasts: after heat shock RT1 and minor histocompatibility antigen-specific CML were reduced. Without *in vivo* priming for target cell antigens or when a major histocompatibility complex (MHC) class I-negative Y3 mutant was used, no lysis was observed, irrespective of heat shock treatment. These findings indicate that the effect of heat shock on target cells depends on their nature and could be suggestive of an involvement of hsp68 in MHC class I-restricted target recognition or killing. Hsp68 transfectants of and hsp68 positive hybrids with the Y3 cell line might be useful for further analysis.

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C.6 Presentation of processing-dependent antigens to MHC class II-restricted T cells is blocked by interference with carbohydrate chain maturation in accessory cells

G. Gradehandt, C. Lobron, K. Reske, and E. Rüde

Treatment of antigen-presenting cells (APC) with tunicamycin which blocks the N-glycosylation of proteins was found to inhibit the presentation of processing-dependent antigens in combination with MHC class II-molecules. Since under these conditions newly synthesized lysosomal enzymes lack mannose-6-phosphate (Man-6-P) residues which serve as a sorting signal for their transport into endosomes and lysosomes we tested the influence of various inhibitors of glycoprotein trimming that still allow Man-6-P attachment on antigen presentation. We found that the inhibition of glucosidase(s), of mannosidase I and mannosidase II which are known to produce oligosaccharide side chains of the core, high mannose, and hybrid type, respectively, interferes with the presentation of processing-dependent antigens such as insulin, ovalbumin, and conalbumin. On the other hand, T-cell recognition of processing-independent peptides was not impaired when the APC lacking mature N-linked oligosaccharide side chains were fixed before adding the peptide to the stimulation culture. Furthermore, the efficiency of peptide presentation was diminished when the inhibitor-treated APC were pulsed with the peptide before fixation. Thus, using this experimental protocol it is concluded that only peptides directly bound to cell surface exposed MHC-molecules are presented irrespective of their glycosylation state, whereas peptide molecules taken up by the APC similar to intact proteins cannot be presented by inhibitor-treated APC.

Institut für Immunologie der Joh.-Gutenberg-Universität, D-6500 Mainz, FRG

C.7 Function of the endo/lysosomal protease Cathepsin B in antigen processing

G. Gradehandt, S. Milbradt, and E. Rüde

The antigens conalbumin (CA) and ovalbumin (OvA) need uptake by antigen-presenting cells (APC) for presentation to MHC class II-restricted T-cells. Both antigens are known to require proteolytic cleavage as a necessary step in the generation of the antigenic peptide. A specific inhibitor of the endosomal protease Cathepsin B, Cbz-Phe-Ala-CHN_2, blocks the presentation of both antigens by APC, whereas this inhibitor has no effect on the presentation of an 18-amino acid OvA peptide. Furthermore, the presentation of insulin, an antigen that needs processing by APC but apparently not by proteolytic cleavage, is enhanced when Cbz-Phe-Ala-CHN_2 is present during antigen-pulsing of the APC. Extracellular digestion of CA with Cathepsin B generates peptides that are able to stimulate D10.G4.1 T-cells in combination with aldehyde-fixed APC. On the other hand, no antigenic peptides could be generated by an 8 h treatment of OvA with Cathepsin B in spite of the OvA-cleavage observed by gel electrophoresis of the digests. This indicates that digestion by Cathepsin B is sufficient to generate the antigenic peptide of CA that is recognized by the D10.G4.1 T cells in combination with I-A^k. On the other hand, Cathepsin B does not suffice to produce the OvA-peptide that stimulates the 3DO-54.8 T cell-hybridoma together with I-A^d. Since treatment of APC with

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Cbz-Phe-Ala-CHN_2 nevertheless prevents presentation of OvA this indicates that the generation of the antigenic OvA-peptide is not a one-step process but involves a second cleavage by another protease, perhaps Cathepsin D, or other alterations as in the case of insulin.

[1]Institute for General and Experimental Pathology, University of Innsbruck, Medical School, Fritz-Pregl-Straße 3, 6020 Innsbruck, Austria, [2]Department of Medical Microbiology, Turku University, Turku, Finland

C.8 Differences in the percentages of lymphocytes bearing $CD4^+$ and $CD8^+$ among chicken congenic lines

Karel Hala[1], Olli Vainio[2], and Günther Böck[1]

Progression and regression of Rous sarcoma virus (RSV) induced tumors are under the control of gene(s) associated with the major histocompatibility complex (MHC). In chickens of the inbred line CB and the recombinant line CB.R1 (shares B-F/B-L region, controlling class I/class II antigens, with line CB) these tumors regressed, while in animals from inbred CC and recombinant CC.R1 lines the tumors progressed. All four congenic lines differ only with regard to the MHC. For further characterization of possible operational mechanism(s) we determined the percentages the T cell subsets, $CD4^+$ and $CD8^+$ in peripheral blood lymphocytes (PBL) by monoclonal antibodies prepared by Vainio et al. By FACScan we found more $CD4^+$ PBL in CB and CB.R1 than in CC and CC.R1. The reverse was true with $CD8^+$ cells-CC had more than CB. The ratio (mean out of 5 animals) of $CD4^+$ to $CD8^+$ in CB chickens was 3.4 and in CC 1.3. The implication of our data for regression or progression of RSV induced tumors will be discussed.

The work was supported by Jubiläumsfonds (No. 3615).

Department of Immunology, University of Kiel, Brunswikerstr. 4, 2300 Kiel, FRG

C.9 Soluble human MHC class I molecules downregulate cytotoxicity of alloreactive CTL

R. Hausmann, N. Zavazava, and W. Müller-Ruchholtz

We investigated the possible interaction of soluble human leukocyte antigen (HLA) class I molecules on alloreactive cytotoxic T lymphocytes (CTL), which play a major role in allograft rejection. HLA-A2 specific CTL-line JS.A2 was generated *in vitro* by coculturing peripheral blood lymphocytes and irradiated Epstein-Barr virus transformed spleen cells. CTL were restimulated every 10–12 days. On third and subsequent restimulations human recombinant interleukin-2 (30 U/ml) was supplemented to the culture medium. Phenotype analysis showed that > 87 % of the cells were $CD8^+$, 9 % $CD4^+$ and 2 % $CD4^+$ $CD8^+$. $CD8^+$ clone 2.G7 was generated by limiting dilution. Soluble HLA class I molecules were isolated from serum or cell lysate by affinity chromatography using the HLA-A2 specific monoclonal antibody PA2.1. Sample purity was analysed by gel electrophoresis and Western blotting. Antigen concentration

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was determined in an indirect enzyme-linked immunosorbent assay. CTL were pre-incubated with purified HLA at concentrations between 0.1 and 1 µg/ml before the cytotoxicity was tested in a ^{51}Cr-release assay. 0.7 µg/ml of purified HLA-A2 molecules induced halfmaximal inhibition, while non-A2 HLA had no effect. Donor-derived HLA in serum of organ recipients was detected in higher concentrations during rejection crises. We speculate that the inhibition of CTL by soluble HLA as described here is of clinical and therapeutical relevance.

Department of Medicine, Joh.-Gutenberg-University, 6500 Mainz, FRG

C.10 Function of the T-cell triggering molecule Tp103 (CD26) in transfected cells

M. Hegen and B. Fleischer

Tp103 is a 103 kD T-cell activation antigen that defines an alternative activation signal for human T lymphocytes. It is absent from or present in only low amounts on resting T-cells but it is expressed strongly after activation. Crosslinking of Tp103 via a mAb CB.1 leads to triggering of functional activities in pre-activated T cells. Tp103 is absent from all T cell leukemia lines tested. We have recently shown that dipeptidylpeptidase IV activity is associated with Tp103 (1).

In this study we have used a cloned cDNA encoding CD26 (kindly provided by D. Camerini, Boston). The cDNA was cloned into the expression vector pKG5 and transfected into several Tp103-negative cell types. Transformants were selected that expressed Tp103 on their surfaces. These cells – but not their non-transfected counterparts – also expressed dipeptidylpeptidase IV activity. This provides the ultimate proof that Tp103 and this enzyme are the same molecules. The transfected molecule was active in the CD3$^+$ Jurkat T-cell leukemia line. The mAb CB.1 stimulated a rise in cytosolic Ca^{++} concentration as well as IL-2 production in these cells. Both responses required crosslinking of the mAb. No response to CB.1 could be elicited in several other cell types expressing the transfected Tp103. These data show that Tp103 can be expressed on transformed T cells and has the same function on these cells as on normal T cells, but is inactive in non-T-cells.

1. Hegen, M. et al. (1990) J. Immunol. 144: 2908.

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[1]Institut für Immungenetik, [2]Innere Klinik und Poliklinik (Tumorforschung), Universitätsklinikum Essen, Hufelandstr. 55, D-4300 Essen, FRG

C.11 Soluble HLA class I and beta-2-microglobulin levels during interferon (IFN) treatment of chronic myelogenous leukemia (CML)

K. Hillebrand[1], T. Moritz[2], U. Westhoff[1], N. Niederle[2], and H. Grosse-Wilde[1]

IFN-α and IFN-γ can induce and augment HLA class I cell-surface expression. Soluble forms of HLA antigens (sHLA-ABC) and of its light chain β-2-microglobulin (β2m) are

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detectable in various body fluids. Recently, we developed an ELISA [Blut 59: 449–454, (1989)] allowing rapid detection of sHLA-ABC. Eleven patients with Ph_1 positive CML in chronic phase were investigated for sHLA-ABC and β2m plasma levels during treatment with IFN (once daily IFN-α-2b s.c. alone or in combination with IFN-γ). Measurements of sHLA-ABC were performed before and 2, 4, 6, 8, 24, 48, and 72 hours after start of treatment and repeated every 2–4 weeks. Baseline sHLA-ABC levels ranged from 13.4 to 42.9 (median 26.2) mg/l. An initial decrease of sHLA-ABC (median 4.6, range 0.7 to 9.1 mg/l) was detected in 9/9 patients tested so far during the first 8 hours. After 24 hours sHLA-ABC levels increased continuously reaching maximum values usually within 3 to 5 weeks of treatment. At this time, increase from baseline sHLA-ABC ranged from 1.0 to 42.2 (median 15.8) mg/l or 4 to 130 (median 76) percent respectively. Thereafter (follow-up 2–7 months) values decreased to near baseline levels in 7/8 patients tested so far. No difference was detected between IFN-α and IFN-α plus IFN-γ treatment. The β2m levels were quantitated in two patients using a commercially available RIA. In both cases, sHLA-ABC and β2m values were highly correlated (r-values 0.82 and 0.84). These preliminary data demonstrate combined changes of sHLA-ABC and β2m in IFN treated CML patients. Possible correlations between HLA-ABC expression on CML cells and clinical courses of CML are currently investigated.

Abt. Angewandte Immunologie, Institut für Radiologie und Pathophysiologie, Deutsches Krebsforschungszentrum (DKFZ), Im Neuenheimer Feld 280, D-6900 Heidelberg, FRG

C.12 A soluble form of lymphocyte function-associated antigen-3 (CD58) detected in normal human sera

Percy Knolle, Reinhard Wallich, and Stefan C. Meuer

Lymphocyte function-associated antigen-3 (LFA-3) CD58 is a broadly distributed cellular adhesion glycoprotein expressed on endothelial, epithelial, connective tissue cells and most cells of hematopoietic origin. CD58 serves as ligand for the T-lymphocyte CD2 glycoprotein. In addition to mediating intercellular adhesion, CD58 can activate T lymphocytes in conjunction with other signals, which is consistent with the ability of pairwise combinations of CD2/CD2R monoclonal antibodies to activate $CD2^+$ cells via the "alternative pathway". CD58 is expressed on the cell surface of nucleated cells in both a polypeptide chain-anchored form and a phosphatidylinositol-anchored form. However, the functional relationship between the two distinct membrane-anchored CD58 forms is still unknown. To study this issue, we have generated a set of monoclonal antibodies directed against different epitopes of the CD58 glycoprotein (A1CD58.1 and A1CD58.2 belonging to the IgG2a subclass and A1CD58.3 of the IgM isotype). Employing the two IgG CD58 monoclonal antibodies a sandwich ELISA system was established. Importantly, a soluble form of CD58 was found to circulate in plasma/serum of healthy individuals (mean concentration: 68 ng/ml SD ± 20). The physiological significance of circulating CD58 is unknown. Presently the functional and physiochemical properties of natural soluble CD58 proteins are under investigation. Given that soluble CD58 may interfere with cellular adhesion processes related to CD2/CD58 interactions it will be necessary to address the question whether altered levels of soluble CD58 exist in clinical disorders of the immune response.

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Klinik für Abdominal- und Transplantationschirurgie Medizinische Hochschule Hannover, 3000 Hannover, und Abteilung Immungenetik der Universität Göttingen, 3400 Göttingen, FRG

C.13 A new loss mutation of the rat major histocompatibility complex

D. Lambracht, W. Wurst, E. Günther, and K. Wonigeit

Two clusters of class I genes are present in the MHC region of the rat (RT1 system); the RT1.A region which codes for classical and the RT1.C region which codes for nonclassical class I antigens. In this study we describe a new mutant haplotype *lm2* with a deletion of expressed RT1.C molecules. The mutant character of *lm2* resulting from an unequal crossing over was demonstrated by comparison of *lm2* with the closely related haplotype *r15* in skin grafting experiments. The haplotype *r15* accepted *lm2* skin indefinitely while acute rejection was observed in the reciprocal combination. The genomic structure of *lm2* was defined by comparing of class I crosshybridizing restriction fragment length polymorphisms of the mutant haplotype *lm2*, the parental haplotypes *u* and *lv3* as well as a panel of recombinant and variant RT1 haplotypes. Major findings from these studies were a) the recombination point within the C region of *lv3* caused a loss of a large part of the C region of *lv3* and a reciprocal gain of C region specific fragments of the other parental hyplotype u; b) missing of two C region fragments shared by the parental haplotypes identified the deleted segment in *lm2;* c) absence of additional bands in *lm2* DNA. The deletion affects different loci as compared to the mutant haplotype *lm1* which we have described previously. The two laboratory derived mutant RT1 haplotypes demonstrate the dynamic structure of the RT1.C region and permit the characterization of multiple antigenic systems encoded by this region of the rat MHC.

Institut für klinische Mikrobiologie der Universität Erlangen/Nürnberg, Wasserturmstraße 3, 8520 Erlangen, FRG

C.14 Analysis of apoptosis induced by triggering of T-cell hybridomas with anti-CD3 antibodies

M. Lohoff, J. Waiser, and M. Röllinghoff

Cell fusion was performed between cells of a T-cell lymphoma line and two different $CD4^+$ long term T-cell clones. The fusion products were tested for their ability to undergo apoptosis after triggering through the T cell receptor. Of 305 independently derived T-cell hybridomas, only one, termed B10, displayed apoptosis after anti-CD3 triggering, whereas 75 % of the hybridomas including B10, produced lymphokines after CD3-triggering, thus demonstrating the ability of the anti-CD3 antibody to stimulate most of the T-cell hybridomas. Apoptosis, as well as lymphokine production of B10 cells was inhibited by cyclosporin A. The involvement of protein kinase C in the process of stimulating apoptosis was demonstrated by the ability of both, inhibitors and stimulators of protein kinase C, to block apoptosis.

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Institute of Medical Microbiology and Hygiene, Technical University of Munich, Trogerstraße 4A, D-8000 Munich 80, FRG

C.15 Exocytosis of serine esterases by cytolytic T cells can be triggered by a variety of T-cell receptor independent stimuli

THOMAS MIETHKE, CLAUDIA WAHL, and HERMANN WAGNER

Exocytosis of serine esterases by cytotoxic T-cells (CTL) is thought to be a critical step during T-cell mediated target cell lysis. To investigate the signal requirements for exocytosis the release of serine esterases of CTL triggered via different stimuli was investigated. Cell sorter purified $CD8^+$ primary resting T cells were incubated with T3-hybridoma cells and IL-2 for seven days. This cell population displayed high lytic activity against T3-hybridoma cells in a ^{51}Cr-release assay; in addition T3-hybridoma cells or CD3 antibodies immobilized on sepharose beads were effective in triggering serine esterases release. However, triggering of these cells via TCR independent stimuli such as hybridoma cells producing antibodies directed against LFA-1, CD2, or CD8 also resulted in an exocytosis of serine esterases. In addition, the same antibodies coupled to sepharose beads efficiently triggered serine esterases release. The data indicate that exocytosis of serine esterases by CTL might occur independent of TCR-triggering.

Institut für Immunologie der Joh.-Gutenberg-Universität, 6500 Mainz, FRG

C.16 Antigen-independent activation of long term-cultured murine T helper cell clones

A. PARTENHEIMER, T. GERMANN, and E. RÜDE

Upon antigen-specific activation T_H cells produce lymphokines and express high affinity IL-2 receptors (IL-2-R). This leads to proliferation by an autocrine mechanism. During a period of 2-3 weeks IL-2-R expression declines, while the T cells revert to a resting state in which they are unresponsive to IL-2. The expression of IL-2-R and proliferation of such T cells cannot only be reinduced by antigen but also in an antigen-independent way by adding allogeneic splenic accessory cells (AC), IL-2 and a partially purified soluble mediator(s) termed TSF. TSF is present in the supernatant of T_H lines stimulated by antigen. For characterization of the role of AC in this stimulation system various cell populations were tested for their ability to replace spleen cells. Furthermore, known cytokines were used in combination with these AC instead of TSF. It was found that in the presence of mast cell lines TSF could not be replaced by other cytokines. However, when using bone marrow derived macrophages cultured with M-CSF, TSF could be replaced by IL-1 and in the presence of freshly isolated bone marrow cells IL-3 or even better, IL-3 + IL-1 were effective similar to TSF. Surprisingly, macrophages cultivated with GM-CSF were stimulatory in the presence of IL-2 alone without additional soluble factors. In order to test whether such GM-CSF-macrophages secrete TSF they were separated from the T cells by a membrane. Under these conditions no proliferation was induced indicating that either cell contact or soluble mediator(s) with short range activity were required.

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Institute for Immunology and Genetics, German Cancer Research Center, Im Neuenheimer Feld 280, 6900 Heidelberg, FRG

C.17 Appearance and instability of "empty" H-2 class I molecules at the cell surface

VIANNEY ORTIZ-NAVARRETE and GÜNTER J. HÄMMERLING

Treatment of the H-2 negative T lymphoma variant RMA-S with K^b and D^b binding NP (nucleoproteins of influenza virus) peptides has been reported to result in the surface expression of the K^b and D^b molecules (1). It has been suggested that the NP peptide facilitates intracellularly the assembly of β2m and the class I heavy chains, and that only this trimolecular complex consisting of β2m, class I chain and peptide can be transported to the cell surface. Strong variations in the surface expression of untreated RMA-S cells prompted us to study the nature of the H-2 molecules on RMA-S in the absence of a specific peptide.

We have observed that in the absence of a peptide the RMA-S cells transport the class I/β2m complex continuously to the cell surface. These K^b and D^b molecules do not seem to contain a peptide and are therefore unstable. However, they can be stabilized by co-cultivation with a ligand, such as anti-class I antibodies or NP peptides. So far, only mAb against the α1 and α2 domains of K^b and D^b could stabilize the class I molecules, but not a mAb against α3, or a mAb against β2m. Maximum level of the "empty" H-2 molecules was observed after about 6 hours. The empty class I molecules appear also at 25 °C at the cell surface and are relatively stable at this temperature, but a temperature shift to 37 °C leads to their disappearance or denaturation within 2 hours.

These observations demonstrate that "empty" class I/β2m molecules can continuously appear at the cell surface of RMA-S cells and that stabilization by a ligand is necessary. It is not yet clear which forces or molecular mechanisms inside the cell stabilize the bimolecular class I/β2m complex in the absence of a peptide.

1. TOWNSEND et al. Nature 340: 443 (1989).

Abt. Angewandte Immunologie, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 6900 Heidelberg, FRG

C.18 A serine phosphatase is involved in CD2-mediated T-cell activation

YVONNE SAMSTAG, ANDREA BADER, and STEFAN C. MEUER

Activation of resting T lymphocytes through the TCR/CD3-complex or, alternatively, the CD2 molecule in both cases leads to a cascade of intracellular events like phosphoinositol breakdown, a rapid rise in cytoplasmic free calcium and activation of protein kinases resulting in phosphorylation of cellular proteins. However, the interrelationship and interdependence of the signal transducing mechanisms of these two pathways of T-cell activation is still unknown. We have investigated early activation events following T cell triggering via TCR/CD3 as compared to activation via CD2. To this end, resting peripheral human T lymphocytes were preincubated with ^{32}P-orthophosphate and subsequently exposed to mitogenic monoclonal antibodies (mAbs) directed at either TCR/CD3 or CD2 for varying time periods. After cold washes cells were lysed and postnuclear lysates subjected to 2D-gel electrophoresis (IEF and

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SDS-PAGE). As early as ten minutes following stimulation through CD2, dephosphorylation of a cytosolic 19 kD protein was observed. In contrast, this protein remained strongly phosphorylated in unstimulated as well as CD3 activated T-cells. pp19 dephosphorylation was transient since, at later time points (2–4 h) following CD2 triggering, this protein was phosphorylated again. Phosphoaminoacid analysis indicated that pp19 is dephosphorylated on serine residue(s). Identical results were obtained employing a CD2 positive/CD3-TCR negative human NK cell clone indicating that pp19 dephosphorylation occurs independent of surface expression of CD3. These data show that, besides protein phosphorylation events, serine dephosphorylation is involved in T cell triggering. More important, a selective signalling mechanism appears to be linked to T cell activation through the CD2 pathway.

Department of Medicine, Division of Immunology and Rheumatology, Stanford University Medical Center S021, Stanford, CA 94305, U.S.A.

C.19 Discrimination between adhesion and co-receptor function of CD4 and CD8

Paul von Hoegen, M. Carrie Miceli, and Jane R. Parnes

CD4 and CD8 are known to be involved in antigen-specific T-cell recognition and activation, however, their mechanism of action are not well understood. It has been suggested that they may function simply as adhesion molecules to strengthen cell-cell interactions, or that they may be involved in signal transduction. Furthermore, CD4 and CD8 may bind their respective major histocompatibility complex (MHC) ligand independently or in concert with the T cell receptor (TCR). We have previously shown that IL-2 secretion by the MHC class II restricted $CD4^+$ and $CD8^-$ T cell hybridoma B1-|4| is dramatically enhanced by expression of CD4, which can bind to the same MHC protein as the TCR. Expression of CD8α, which cannot bind to the same MHC protein as the TCR, also augments IL-2 production by B1-|4|, although much less well than CD4. Transfection of CD8α', which lacks a cytoplasmic tail and is not associated with the intracellular tyrosine kinase $p56^{lck}$, enhances IL-2 production to the same degree as CD8α. Taken together with previous results, these data suggest that the ability of the CD8α cytoplasmic tail and/or the associated $p56^{lck}$ to potentiate T cell activation is a "co-receptor function" dependent on binding to the same ligand as the TCR.

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Workshop D
T Lymphocytes, Microenvironment and Autoimmunity

II. Medizinische Klinik, Technische Universität, Ismaninger Straße 22, D-8000 München 80, FRG

D.1 The CD45 molecule differentially regulates the activation of human intestinal lamina propria lymphocytes induced by CD3 crosslinking

K. Deusch, F. Lüling, K. Reich, and M. Classen

The CD45 molecule is a transmembrane protein expressed on virtually all hematopoetic cells and has been shown to exert significant tyrosine-phosphatase activity. Several isoforms of this molecule are differentially expressed in T lymphocytes and this behavior appears to correlate with the functional activity of these cells. In this study, we were interested as to whether antibodies directed to CD45, CD45RO or CD45RA can influence the activation of human colonic lamina propria T lymphocytes (LPL) upon crosslinking to the CD3/TCR-complex employing suboptimal doses of anti-CD3 antibodies coated onto plastic dishes. We found that anti-CD45RO antibody (UCHL-1) most potently enhanced the proliferative response of LPL, stimulation index (S.I.) = 65. Surprisingly, anti-CD45 antibody that recognizes all forms of CD45 augmented LPL-proliferation to a much lesser extent (S.I. = 18). Anti-CD45RA antibodies had only little effect (S.I. = 3). However, this may be due to the low frequency of these cells within the LPL-population in the lamina propria (< 15 %). Immunohistochemical and FACS analysis revealed that almost 90 % of LPL cells expressed both CD45 and CD45RO. Therefore, we conclude that our data support the notion that the CD45RO molecule plays a distinct functional role during the activation processes incited by the perturbation of the T-cell antigen receptor complex expressed on human lamina propria T-lymphocytes.

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[1]II. Medizinische Klinik und [2]Institut für Mikrobiologie und Hygiene, Technische Universität, Ismaninger Straße 22, D-8000 München 80, FRG

D.2 A distinct set of human gamma/delta (TCR1$^+$) cells homes to the intestinal epithelium

K. Deusch[1], K. Reich[1], F. Lüling[1], K. Pfeffer[2], and M. Classen[1]

We have found that TCR1$^+$ T lymphocytes preferentially home to the human intestinal epithelium. The majority of these cells were shown to express CD8 molecules on their surface.

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In this study, we demonstrate that, in normal human colonic epithelium, TCR1$^+$ intraepithelial lymphocytes (IEL) represent a distinct subset of T-cells using a restricted set of variable region genes to synthesize their γ/δ-T-cell receptor (TCR1). Employing monoclonal antibodies directed to the surface products of defined variable regions and subsequent FACS analysis, we will show that TCR1$^+$-IEL are markedly restricted to the use of the V-delta-1 region (64 %) assembling their TCR-complex. In marked contrast, peripheral blood-TCR1$^+$ cells obtained from the same individuals preferentially expressed the product of the V-gamma-9/V-delta-2 (79 %) region on their surface. The frequency of TCR1$^+$ cells in the lamina propria (LPL) using V-delta-1 or V-gamma-9 antibodies were 42 % and 49 %, respectively, which indicates that these cells represent a "transitional" population. The products of V-delta-1 and V-gamma-9 represent polypeptides that have been described to not associate to form a functional TCR1-complex and therefore appear to delineate distinct sets of TCR1$^+$ cells. Taken together, our findings clearly demonstrate that TCR1$^+$-IEL not only differ from TCR1$^+$-PBL with regard to CD8 expression but also, and perhaps more importantly, by virtue of distinct usage of TCR-variable regions. In conclusion, these data suggest that a unique subset of TCR1$^+$ cells exhibiting a restriced receptor repertoire home to the colonic intestinal epithelium. Alternatively, V-delta-1 cells could have been locally expanded due to the presence of unique antigens on the luminal surface of the intestine that are normally excluded from the peripheral blood.

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Institute for Immunology, University of Munich, Goethestraße 31, 8000 München 2, FRG

D.3 T cells express the B isoform of FcεRII after stimulation with allergen or PHA

N. Endres, J. C. Prinz, and E. P. Rieber

For the low affinity IgE receptor CD23 two isoforms have been described that differ in the six N-terminal cytoplasmic amino acids. FcεRIIa is constitutively expressed on B cells, while the expression of FcεRIIb can be selectively induced by IL-4 in B cells and monocytes (1). CD23 is also found on few T cells after PHA stimulation, whereas a major portion of T blasts express CD23 when PBL from allergic individuals are activated by the specific allergen (2). In order to characterize the CD23 isoforms expressed in T cells, PBL from normal and allergic individuals were stimulated, the T cells were purified at the time of maximal CD23 expression, RNA was isolated, transcribed into cDNA and analysed by PCR with CD23a- or b-specific primers. Only FcεIIb could be detected in purified T cells activated either by PHA or in T cells from allergic donors stimulated with the specific allergen. No CD23 message was identified by PCR in ConA- or tetanus toxoid-induced T blasts. FcεIIa was never found in T cells. IL-4 alone was not capable to induce CD23 on T cells, but was found to enhance the expression of FcεRIIb after stimulation with PHA or allergen.

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[1]Abt. für Immunologie, 1. Medizinische Klinik, Universitätskrankenhaus, 2000 Hamburg, FRG, and [2]Department of Pathology, Stanford University, U.S.A.

D.4 Role of adhesion molecules in lymphocyte migration

A. Hamann[1], Cornelia Berlin[1], B. Holzmann[2], Andrijka Kashan[1], Dorothee Jablonski-Westrich[1], and H.-G. Thiele[1]

Selective immigration of lymphocytes into different organs seems to be regulated by different adhesion molecules. Inhibitory effects of monoclonal antibodies on *in vitro* binding of lymphocytes to high endothelial venules (HEV) in the frozen section assay have led to the postulation of at least two types of "homing receptors": a peripheral node-specific receptor, represented in the mouse by $gp90^{MEL-14}$, and a Peyer's patch (PP)-specific, for which the integrin LPAM 1/2 has been proposed to be a candidate. Additional molecules like LFA-1 are supposed to play a more general accessory role in lymphocyte-endothelium interactions. Our data show some organ-specific effects in migration for this molecule also. *In vivo* homing studies indicate that the functional role of the "homing receptors" is still far from being clear. MEL-14 antibodies *in vivo* also inhibit the immigration of lymphocytes into PP, albeit only partially. It is still unclear, whether $gp90^{MEL-14}$ may also recognize PP-HEV *in vivo*, or whether this finding points to additional functions of $gp90^{MEL-14}$ in the regulation of general adhesion events. Antibodies against LPAM 1/2 inhibit *in vitro* binding to PP-HEV, but in first experiments, no significant inhibition was seen for homing into PP *in vivo*. Further complications arise in the interpretation of the migration patterns of activated lymphocytes. Their behavior cannot sufficiently be explained by the function (or lack of function) of one of these molecules. Binding studies using transformed mouse endothelial cell lines point to the relevance of further, so far unidentified interaction molecules. Data from this experimental model confirm findings in the human system, showing an upregulation of binding mechanisms on endothelium by inflammatory mediators like TNF.

Institute for Clinical Chemistry and Pathobiochemistry, RWTH Aachen, Pauwelsstraße, D-5100 Aachen, FRG

D.5 Analysis of activated T-cell populations in synovial fluid of patients with rheumatoid arthritis

H.-D. Haubeck

Several lines of evidence suggest the involvement of specific autoreactive T lymphocytes in the pathogenesis of rheumatoid arthritis (RA). In the synovium of patients with rheumatoid arthritis dense infiltrates of mononuclear cells (lymphocytes, monocytes) can be found. A major subpopulation of these infiltrating cells are $CD4^+$ T-helper (Th) cells which can be shown to express activation antigens (Ag). The concept that autoreactive Th cells are involved in the induction and perpetuation of the inflammatory process in RA is further supported by the marked HLA disease association.

By using a panel of monoclonal antibodies (mAb's) we have analyzed the expression of activation Ag's on T lymphocytes of synovial fluid from patients with RA. For a detailed analysis mAb's for differentiation Ag's, activation Ag's, Ag's of the different T cell receptors (TCR alpha, beta and TCR gamma, delta) and of memory T-cells were used. Usually 80–90 %

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of the lymphocyte population were of the T cell phenotype. About 95 % of these T cells used the classical T cell receptor (TCR alpha, beta) whereas only 1–5 % used the alternate receptor (TCR gamma, delta). The percentage of $CD4^+$ Th cells ranged from 20–60 %. The expression of activation antigens varied from 0–60 % depending on mAb's for the different activation Ag's. About 80–90 % of the lymphocytes expressed antigens of memory cells (CD 29, CD 45RO).

Activated $CD4^+$ Th cells will now be sorted and used in antigen-dependent T-cell proliferation assays for isolation and characterization of antigens which are responsible for the induction and maintenance of the chronic autoimmune process in RA.

Division of Clinical Immunobiology, Department of Internal Medicine and Institute of Blood Transfusion, University of Innsbruck, Innsbruck, Institute for Blood Group Serology, Vienna, Austria, and Department of Internal Medicine III, Ludwig-Maximilians-University, Munich, FRG

D.6 Cytotoxic lymphocyte precursor (CTL-p) frequencies in allogeneic bone marrow recipients

E. Irschick, F. Hladik, D. Niederwieser, D. Schönitzer, A. Hajek, E. Holler, H. J. Kolb, and Ch. Huber

CTL-p frequencies were determined by limiting dilution analysis in twelve recipients of allogeneic HLA sibling bone marrow grafts. Eleven patients received HLA identical grafts, one had one HLA-DR mismatch. Tests were set up in graft-versus-host (GvH) direction. Recipient-pre-transplantation (Tx) specific and thirdparty reactive CTL-p were assessed before and one month after grafting or at the time of GvH-disease (GvH-D). Additionally, split-well analyses were performed and tested against recipient-pre-Tx and donor targets.

Very low recipient-pre-Tx specific frequencies were detected in two out of five patients without GvH-D. Six patients developed GvH-D but only one patient with a severe GvH-D showed a high recipient-pre-Tx specific CTL-p frequency at the time of disease. Third-party specific CTL-p frequencies were detectable in all patients and were significantly reduced in eleven of twelve cases after transplantation. Donor specific CTL-p were not detected at any tested time point in split-well analysis.

Max-Planck-Society, Clinical Research Unit of Rheumatology/Immunology; Department of Nuclear Medicine; University of Erlangen–Nürnberg, 8520 Erlangen, FRG

D.7 T-cell migration in rat adjuvant arthritis

R. Kinne, H. Reinecke, S. Bloch, U. Vorderwülbecke, W. Becker, and F. Emmrich

The relevance of T cells for the induction and perpetuation of rat adjuvant arthritis (AA) has been demonstrated by a) adoptive transfer of AA by T cells and b) successful treatment of AA with anti-T-cell monoclonal antibodies. However, little is known about the kinetics of T-cell migration into arthritic joint tissue in AA.

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